


CLAIMS:

1. A biochip comprising:
 - a) a solid substrate having a surface;
 - b) at least one optically clear hydrogel cell attached to the surface of the substrate, which hydrogel cell is formed from an isocyanate-functional polymer; and
 - c) a binding entity immobilized within or upon said hydrogel cell, which entity is effective to selectively hybridize to or sequester a target molecule.
2. The biochip of claim 1 wherein the hydrogel comprises a polymer with urethane linkages.
3. The biochip of claim 1 wherein the hydrogel comprises polyethylene glycol, polypropylene glycol, or copolymers thereof.
4. The biochip of claim 1 wherein the hydrogel cell is at least 20 μm thick.
5. The biochip according to claim 4, wherein the hydrogel cell is between about 30 μm and about 100 μm thick.
6. The biochip according to claim 1, wherein said binding entity is covalently bound to and within the hydrogel cell through reaction with isocyanate groups.
7. The biochip of claim 5 wherein about 15% or less of the reactive isocyanates in said polymer of said cell have reacted with said binding entities.
8. The biochip of claim 1 wherein with said binding entity comprises DNA, RNA or PNA.

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9. The biochip of claim 1 wherein said binding entity comprises an immunoglobulin, an enzyme, a receptor, an enzyme inhibitor, an enzyme substrate, or a peptide.
10. The biochip of claim 9 wherein each said binding entity is immobilized within the hydrogel through an interaction with an intermediate agent
11. The biochip of claim 1 wherein said binding entity is a protein which is bound to a metal chelate that is immobilized in the hydrogel and constitutes said intermediate agent.
12. The biochip of claim 11 wherein said protein is bound to said metal chelate through a histidine-containing polypeptide at one terminal of said protein.
13. The biochip of claim 1 wherein said binding entity is immobilized through a first intermediate agent linked to the hydrogel and a second intermediate agent linked to said first intermediate agent.
14. The biochip of claim 13 wherein said first intermediate agent is an antibody and the second intermediate agent is a protein.
15. The biochip of claim 1 wherein the substrate has a plurality of hydrogel cells attached to its surface and wherein different binding entities are immobilized in different hydrogel cells.
16. The biochip of claim 5 wherein said surface has a plurality of wells formed therein.
17. The biochip of claim 1 wherein the substrate is optically transparent and has reactive molecules on its top to which the hydrogel is covalently bound through some of said isocyanate groups of the polymer.

18. A hydrogel biochip comprising:

- a) a solid substrate having a top surface;
- b) a plurality of hydrogel cells comprising polyethylene glycol, polypropylene glycol, or copolymers thereof bound to the top surface of said substrate;
- c) intermediate agents immobilized within or upon said hydrogel of said cells; and
- d) different protein binding entities bound to said intermediate agents within at least several of said hydrogel cells by interaction therewith in a manner so that said protein binding entities assume their native conformations.

19. A method of using a biochip to carry out a biochemical assay, which method comprises the steps of:

- (a) providing an optically clear hydrogel biochip having a substrate with a surface to which at least two hydrogel cells are bound, each cell having a thickness of at least about 20 μm and being predominantly comprised of polyethylene glycol, polyethylene glycol or a copolymer thereof, each said hydrogel cell including a different binding entity immobilized therewithin or thereupon,
- (b) contacting the hydrogel biochip with an analyte solution, containing a target biomolecule under binding conditions;
- (c) washing the hydrogel biochip under conditions that remove non-selectively bound and unbound target biomolecule; and
- (d) detecting the target biomolecule bound to one of said cells.

20. The method of claim 19 wherein binding of target biomolecule results in a compositional change of the binding entry in the form of a phosphorylation event or dephosphorylation event.

21. A method of preparing an optically clear isocyanate-functional hydrogel biochip having a binding entity immobilized therewithin or thereon, which entity is effective to selectively sequester or hybridize to a target biomolecule, the method comprising the steps of:

- a) providing an organic solvent solution of an isocyanate-functional hydrogel prepolymer;
- b) providing a solution of said binding entity;
- c) covalently binding said entity to the isocyanate-functional hydrogel prepolymer via reaction with not more than 15% of said reactive isocyanates;
- d) initiating polymerization of the isocyanate-functional hydrogel prepolymers under conditions that will produce an optically clear hydrogel; and
- e) dispensing the polymerizing isocyanate-functional hydrogel prepolymer in droplet form onto a solid substrate, such that an optically clear hydrogel polymer containing said binding entity is attached to said substrate.

22. The method of claim 21 wherein the binding of said entity is performed simultaneously with polymerization.

23. The method of claim 10 wherein viscosity and pH are selected to control carbon dioxide evolution to assure clarity of the resulting hydrogel and said substrate is treated to provide reactive moieties on its top surface which will covalently bind the polymerizing hydrogel to said substrate.

24. A method of preparing an isocyanate-functional hydrogel biochip having proteins immobilized therein or thereupon which are chosen to function as capture agents, the method comprising the steps of:

- a) providing an organic solvent solution of an isocyanate-functional hydrogel prepolymer;
- b) providing solutions of desired protein capture agents;
- c) covalently binding intermediate coupling agents for said proteins to the isocyanate-functional hydrogel prepolymer;
- d) initiating polymerization of said isocyanate-functional hydrogel prepolymer;
- e) dispensing droplets of the polymerizing isocyanate-functional hydrogel prepolymer onto a solid substrate, such that said polymer becomes attached to said substrate; and

f) exposing individual hydrogel droplets to one of said desired protein solutions to immobilize said protein capture agents therein or thereupon via connection to said coupling agents,

whereby said droplets polymerize to create a biochip having a plurality of cells with different protein captive agents.

25. The method of claim 24 wherein said connection of said proteins to said coupling agent is performed simultaneously with polymerization.

26. The method of claim 24 wherein said connection of said proteins to said coupling agent is performed subsequent to polymerization.

27. The method of claim 24 wherein said coupling agent is a chelating agent and the proteins each include a histidine-containing terminal peptide sequence.

28. The method of claim 24 wherein reaction conditions during said polymerizing are controlled to slow the rate of carbon dioxide evolution and assure optical transparency of the resulting hydrogel and wherein said substrate is treated to provide reactive moieties on its top surface that will covalently bind said hydrogel to said substrate.

29. A method of preparing an isocyanate-functional hydrogel biochip having a plurality of cells which have binding agents immobilized therein or thereupon, the method comprising the steps of:

a) providing an organic solvent solution of an isocyanate-functional hydrogel prepolymer;

b) initiating polymerization of the isocyanate-functional hydrogel prepolymer;

c) dispensing droplets of the polymerizing isocyanate-functional hydrogel prepolymer onto a solid substrate so that said droplets become attached to said substrate and form of a plurality of cells; and

d) physically immobilizing a different binding agent in or upon each of at least two of said cells, said binding agents being chosen to hybridize to or selectively sequester a particular biomolecule.

30. The method of claim 29 wherein said binding agents are proteins having a molecular weight of about 100,000 or greater and said immobilizing comprises physical entrapment of the proteins via the use of electrical current to cause the proteins to diffuse into said cells.